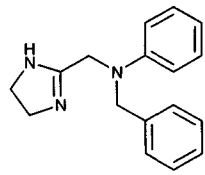
Antazoline



Molecular formula: $C_{17}H_{19}N_3$
Molecular weight: 265.36
CAS Registry No.: 91-75-8, 2508-72-7
Merck Index: 718
Lednicer No.: 1 242

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.6

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl,

protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 11.04

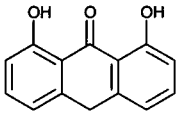
OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Anthralin



Molecular formula: $C_{14}H_{10}O_3$
Molecular weight: 226.23
CAS Registry No.: 1143-38-0
Merck Index: 723

SAMPLE

Matrix: bulk
Sample preparation: Prepare a 1 mg/mL solution in dichloromethane. Mix a 1 mL aliquot with 19 mL dichloromethane and 1 mL acetic acid, make up to 50 mL with hexane. Inject an aliquot.

HPLC VARIABLES

Guard column: 4 × 4 5 μm LiChrospher 100 RP18
Column: 250 × 4 5 μm LiChrospher 100 RP18
Mobile phase: MeOH:water:acetic acid 77:23:0.1, adjusted to pH 5.5 with conc. ammonia
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 15.6

OTHER SUBSTANCES

Simultaneous: impurities

REFERENCE

Müller,K.; Ziereis,K.; Wiegrebe,W. The monograph dithranol in the European Pharmacopoeia -comment and amendments, *Pharmazie*, **1996**, *51*, 980–981.

SAMPLE

Matrix: formulations
Sample preparation: Creams, gels, lotions. Disperse and dilute in MeCN to an anthralin concentration of 0.25 mg/mL. Remove a 10 mL aliquot and add it to 10 mL 1 mg/mL anthracene in MeCN, 10 mL chloroform, 20 mL MeCN, and 0.25 mL acetic acid. Filter (Whatman No. 42 paper), inject a 10 μL aliquot. Sticks, ointments. Disperse and dilute in chloroform to an anthralin concentration of 0.25 mg/mL. Remove a 10 mL aliquot and add it to 10 mL 1 mg/mL anthracene in MeCN, 30 mL MeCN, and 0.25 mL acetic acid. Filter (Whatman No. 42 paper), inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water:acetic acid 60:39.5:0.5 containing 0.05% sodium hexanesulfonate
Flow rate: 2.5
Injection volume: 10
Detector: UV 365

CHROMATOGRAM

Retention time: 6.5
Internal standard: anthracene (8.5)

OTHER SUBSTANCES

Simultaneous: danthron, dianthrone

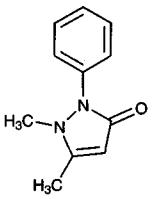
KEY WORDS

protect from light; deaerate all solvents with argon; creams; gels; lotions; sticks; ointments

REFERENCE

Burton,F.W.; Gadde,R.R. Analysis of anthralin in dermatological products by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *328*, 317–324.

Antipyrine



Molecular formula: $C_{11}H_{12}N_2O$
Molecular weight: 188.23
CAS Registry No.: 60-80-0, 520-07-0 (salicylate)
Merck Index: 757
Lednicer No.: 1 234

SAMPLE

Matrix: amniotic fluid, blood
Sample preparation: Condition a 3 mL Bond Elut C18 SPE cartridge with 2 mL MeOH and 2 mL water. 50 μL Plasma or amniotic fluid + 50 μL 5 μg/mL 3-hydroxyacetamidophenol in water, add to SPE cartridge, wash twice with 2 mL portions of water, elute with 2 mL MeOH. Evaporate the eluate under vacuum, reconstitute the residue in 100 μL MeCN:water 6:94, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere ODS C18
Mobile phase: Gradient. A was MeCN:pH 4.0 ammonium phosphate buffer 6:94. B was MeCN:pH 4.0 ammonium phosphate buffer 25:75. A:B from 100:0 to 100:0 over 20 min, to 100:0 over 5 min, re-equilibrate for 10 min.
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Limit of quantitation: 400 ng/mL

OTHER SUBSTANCES

Extracted: didanosine

KEY WORDS

plasma; monkey; pharmacokinetics; SPE

REFERENCE

Pereira,C.M.; Nosbisch,C.; Winter,H.R.; Baughman,W.L.; Unadkat,J.D. Transplacental pharmacokinetics of dideoxyinosine in pigtailed macaques, *Antimicrob.Agents Chemother.*, **1994**, *38*, 781–786.

SAMPLE

Matrix: amniotic fluid, blood
Sample preparation: Condition a 3 mL Bond Elut C18 SPE cartridge with 2 mL MeOH and two 2 mL portions of water. 100 μL Plasma or amniotic fluid + 20 μL 10 μg/mL 3-hydroxyacetamidophenol in water, mix, add to the SPE cartridge, wash with two 2 mL portions of water, elute with 2 mL MeOH. Evaporate the eluate to dryness under reduced pressure, reconstitute with 100 μL MeCN:50 mM pH 3.3 ammonium phosphate buffer 6:94, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. A was MeCN:50 mM pH 3.3 ammonium phosphate buffer 6:94. B was MeCN:50 mM pH 3.3 ammonium phosphate buffer 25:75. A:B from 100:0 to 0:100 over 17 min, maintain at 0:100 for 5 min, return to initial conditions over 3 min, re-equilibrate for 17 min.
Flow rate: 1
Injection volume: 50

Detector: UV 266

CHROMATOGRAM
Limit of quantitation: 400 ng/mL

OTHER SUBSTANCES
Extracted: stavudine

KEY WORDS
monkey; plasma; pharmacokinetics; SPE

REFERENCE
Odinecs,A.; Nosbisch,C.; Keller,R.D.; Baughman,W.L.; Unadkant,J.D. In vivo maternal-fetal pharmacokinetics of stavudine (2',3'-didehydro-3'-deoxythymidine in pigtailed macaques (*Macaca nemestrina*), *Antimicrob.Agents Chemother.*, **1996**, *40*, 196–202.

SAMPLE
Matrix: blood
Sample preparation: 0.5 mL Plasma + 0.5 mL water + 0.5 mL 0.25 M NaOH, vortex, allow to stand at room temperature for 20 min, add 20 μL of 100 μg/mL phenacetin and 3 μg/mL flunitrazepam, vortex, add 5 mL diethyl ether, vortex for 30 s, centrifuge at 900 g for 5 min, freeze in acetone/dry ice for 5 min. Remove the supernatant and dry it under nitrogen. Reconstitute in 115 μL MeCN:0.1% pH 3 sodium phosphate buffer 30:70, inject an aliquot.

HPLC VARIABLES
Guard column: 23 × 3.9 37-50 μm μBondapak phenyl
Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: Gradient. A was MeCN:0.1% pH 3 sodium phosphate buffer 5:95. B was MeCN:0.1% pH 3 sodium phosphate buffer 70:30. A:B 80:20 for 2.5 min, then to 45:55 over 20 min, then to 25:75 over 3 min, then to 80:20 over 3 min, equilibrate at 80:20 for 7 min.
Column temperature: 40
Flow rate: 2
Injection volume: 200
Detector: UV 254

CHROMATOGRAM
Retention time: 5.08
Internal standard: phenacetin (7.09)
Limit of quantitation: 5500 ng/mL

OTHER SUBSTANCES
Simultaneous: lorazepam (at 229 nm)
Noninterfering: acetaminophen, trimethoprim, sulfamethoxazole, allopurinol, indocyanine green

KEY WORDS
plasma

REFERENCE
Riley,C.A.; Evans,W.E. Simultaneous analysis of antipyrine and lorazepam by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *382*, 199–205.

SAMPLE
Matrix: blood

Sample preparation: 0.5 mL Plasma +10 μL 250 μg/mL 1-acetamidopyrene in MeOH + 200 μL 1 M ammonium sulfate + 800 μL cold MeCN, vortex for 30 s, store at -20° for at least 30 min, vortex, centrifuge at 1500 g for 30 min. Remove 400 μL of the upper organic layer and evaporate it under a stream of nitrogen. Reconstitute with 100 μL mobile phase, vortex for 30 s, inject a 75 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:buffer 47:53 (Buffer was 6.805 g potassium monophosphate in 1 L water, adjust pH to 6.00 with 10 M NaOH.)
Flow rate: 1
Injection volume: 75
Detector: UV 214

CHROMATOGRAM

Retention time: 4.2
Internal standard: 1-acetamidopyrene (9.7)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Simultaneous: lorazepam, indocyanine green
Noninterfering: adenosine, albuterol, alphenal, aspirin, caffeine, carbamazepine, cefazolin, cephalexin, cephalothin, cimetidine, ciprofloxacin, claforan, desipramine, enoxacin, fleroxacin, furosemide, hydralazine, hydrochlorothiazide, minoxidil, norfloxacin, phenytoin, propafenone, sulindac, teicoplanin, theophylline, vancomycin
Interfering: some indocyanine green impurities

KEY WORDS

plasma

REFERENCE

Awni,W.M.; Bakker,L.J. Antipyrine, indocyanine green, and lorazepam determined in plasma by high-pressure liquid chromatography, *Clin.Chem.*, **1989**, *35*, 2124–2126.

SAMPLE

Matrix: blood
Sample preparation: Make plasma alkaline with NaOH, extract with dichloromethane.

HPLC VARIABLES

Column: reverse phase
Mobile phase: MeOH:water 43:57 containing 100 mM triethylamine, adjusted to pH 4.7 with orthophosphoric acid
Detector: UV 254

CHROMATOGRAM

Internal standard: phenacetin
Limit of detection: 100 ng/mL

KEY WORDS

plasma

REFERENCE

Hayton,W.L.; Kneer,J.; Blouin,R.A.; Stoeckel,K. Pharmacokinetics of intravenous cefetamet and oral cefetamet pivoxil in patients with hepatic cirrhosis, *Antimicrob.Agents Chemother.*, **1990**, *34*, 1318–1322.

SAMPLE

Matrix: blood

Sample preparation: 200 μL Serum + 200 μL mobile phase, filter (Millipore Millex 0.45 μm), inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 40 × 4 C18 Corasil II
Column: 300 × 4 10 μm μBondapak phenyl
Mobile phase: Propanol:6 mM C_{12} DAPS (Fluka) 3:97 (C_{12} DAPS is 3-(dimethyldodecylammonio) propanesulfonate.)
Injection volume: 25
Detector: UV 273

CHROMATOGRAM
Retention time: 8
Internal standard: antipyrine

OTHER SUBSTANCES
Simultaneous: theophylline, β-hydroxytheophylline, caffeine, theobromine, albendazole, albendazole sulfoxide, nimorazole, flubendazole, mercaptopurine, aminophylline, amyleine, procaine
Interfering: metronidazole, tinidazole, dipropyline

KEY WORDS
serum; micellar chromatography; antipyrine is IS

REFERENCE
Habel,D.; Guermouche,S.; Guermouche,M.H. Direct determination of theophylline in human serum by high-performance liquid chromatography using zwitterionic micellar mobile phase. Comparison with an enzyme multiplied immunoassay technique, *Analyst*, **1993**, *118*, 1511–1513.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 1 μg phenacetin + 100 μL 100 mM NaOH + 400 μL water + 3 mL dichloromethane, shake vigorously for 15 min, centrifuge at 1500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 7 μm Hibar LiChrosorb RP-18
Mobile phase: MeCN:10 mM pH 8 phosphate buffer 20:80
Flow rate: 1.5
Injection volume: 40
Detector: UV 254

CHROMATOGRAM
Retention time: 6
Internal standard: phenacetin (12)
Limit of detection: <500 ng/mL

KEY WORDS
plasma

REFERENCE
Wolfisberg,H.; Schmutz,A.; Stotzer,R.; Thormann,W. Assessment of automated capillary electrophoresis for therapeutic and diagnostic drug monitoring: determination of bupivacaine in drain fluid and antipyrine in plasma, *J.Chromatogr.A*, **1993**, *652*, 407–416.

SAMPLE
Matrix: blood

Sample preparation: 50 μL Plasma + 500 μL 370 mM pH 4.6 acetate buffer + cyclobarbital + 5 mL dichloromethane, vortex, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 70 μL mobile phase, inject an aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 Cyclobond II (Baker)
Column: 250 × 4.6 Cyclobond II (Baker)
Mobile phase: MeOH:water 15:85
Flow rate: 0.7
Injection volume: 20
Detector: UV 214

CHROMATOGRAM

Internal standard: cyclobarbital
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES

Extracted: hexobarbital, phenobarbital

KEY WORDS

plasma; rat

REFERENCE

Groen,K.; Breimer,D.D.; Jansen,E.J.; Van Bezooijen,C.F.A. The influence of aging on the metabolism of simultaneously administered hexobarbital enantiomers and antipyrine before and after phenobarbital induction in male rats: A longitudinal study, *J.Pharmacol.Exp.Ther.*, **1994**, *268*, 531–536.

SAMPLE

Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL 20 μg/mL cefadroxil + 8 mL dichloromethane, shake for 20 min, centrifuge at 2500 rpm for 20 min. Remove 7 mL of the organic layer and evaporate it to dryness under nitrogen or at 60°. Dissolve residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 6 Shimpack CLS-ODS (Shimadzu)
Mobile phase: MeCN:0.5 mM phosphoric acid 12:88
Column temperature: 40
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Internal standard: cefadroxil

KEY WORDS

plasma; rat

REFERENCE

Lee,C.K.; Uchida,T.; Kitagawa,K.; Yagi,A.; Kim,N.-S.; Goto,S. Skin permeability of various drugs with different lipophilicity, *J.Pharm.Sci.*, **1994**, *83*, 562–565.

SAMPLE

Matrix: blood
Sample preparation: 0.5 mL Plasma + 25 μL 40 μg/mL 3-acetamidophenol extracted with ether:dichloromethane:isopropanol 60:40:1. Evaporate organic layer under a stream of nitrogen and take up residue in 250 μL 50 mM pH 7.8 phosphate buffer.

HPLC VARIABLES
Column: 100 × 4.6 Chrompack microsphere C18
Mobile phase: Gradient. A was 50 mM pH 7.8 buffer. B was MeOH:50 mM pH 7.8 phosphate buffer 50:50. A:B from 100:0 to 50:50 over 38 min, maintain at 50:50 for 2 min.
Flow rate: 0.7
Injection volume: 250
Detector: UV 254

CHROMATOGRAM
Internal standard: 3-acetamidophenol

OTHER SUBSTANCES
Simultaneous: acetaminophen, metabolites

KEY WORDS
plasma; pig

REFERENCE
Monshouwer,M.; Witkamp,R.F.; Pijpers,A.; Verheijden,J.H.M.; Van Miert,A.S.J.P.A.M. Dose-dependent pharmacokinetic interaction between antipyrine and paracetamol *in vivo* and *in vitro* when administered as cocktail in pig, *Xenobiotica*, **1994**, *24*, 347–355.

SAMPLE
Matrix: blood
Sample preparation: 0.5 mL Plasma + 25 μL 40 μg/mL 3-acetamidophenol extracted with ether:dichloromethane:isopropanol 60:40:1. Evaporate organic layer under a stream of nitrogen and take up residue in 50 mM pH 7.8 phosphate buffer, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 Chrompack microsphere C18
Mobile phase: Gradient. A was 50 mM pH 7.8 phosphate buffer. B was MeOH:50 mM pH 7.8 phosphate buffer 50:50. A:B from 100:0 to 50:50 over 38 min, maintain at 50:50 for 2 min.
Flow rate: 0.7
Injection volume: 250
Detector: UV 254

CHROMATOGRAM
Internal standard: 3-acetamidophenol

OTHER SUBSTANCES
Extracted: acetaminophen

KEY WORDS
plasma; metabolites; pig; pharmacokinetics

REFERENCE
Monshouwer,M.; Witkamp,R.F.; Pijpers,A.; Verheijden,J.H.M.; Van Miert,A.S.J.P.A.M. Dose-dependent pharmacokinetic interaction between antipyrine and paracetamol *in vivo* and *in vitro* when administered as cocktail in pig, *Xenobiotica*, **1994**, *24*, 347–355.

SAMPLE
Matrix: blood
Sample preparation: Plasma + phenacetin + 10% trichloroacetic acid, centrifuge, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 100 × 4.6 3 μm Nucleosil C18
Mobile phase: MeCN:buffer 25:75 (Buffer was 50 mM citrate adjusted to pH 5 with acetic acid.)
Column temperature: 50
Flow rate: 1.5
Injection volume: 20
Detector: UV 244

CHROMATOGRAM

Internal standard: phenacetin
Limit of quantitation: 500 ng/mL

KEY WORDS

dog; plasma; pharmacokinetics

REFERENCE

Galtier,M.; Briand,D.; Pinguet,F.; Gomeni,R.; Fabre,D.; Bressolle,F. Pharmacokinetic parameters of antipyrine in dog after hepatectomy, *Biopharm.Drug Dispos.*, **1995**, *16*, 669–684.

SAMPLE

Matrix: blood, saliva
Sample preparation: 1 mL Plasma or saliva + 100 μL 400 μg/mL phenacetin in EtOH + 100 μL 2 M NaOH + 5 mL dichloromethane:n-pentane 50:50, vortex for 15 s. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES

Column: 10 μm Spheri-10 RP-18
Mobile phase: MeCN:100 mM sodium acetate:triethylamine 7.5:92:0.5, pH 6.6
Column temperature: 40
Flow rate: 3.5
Injection volume: 25
Detector: UV 254

CHROMATOGRAM

Internal standard: phenacetin

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Jorquera,F.; Almar,M.M.; Jimeno,A.; González-Sastre,M.; González-Gallego,J. Assessment of antipyrine kinetics from saliva or plasma: influence of age, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1141–1145.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. Mix 500 μL plasma with 25 μL 15 μg/mL IS in MeOH, 500 μL 750 mM pH 5.0 sodium acetate buffer containing 40 mg/mL sodium metabisulfite, and 10 mg of a mixture of β-glucuronidase and arylsulfatase (Limpet acetone powder Type I: Platela vulgata, Sigma). Heat at 37° for 2 h, add 100 mg NaCl, extract with 1.2 mL chloroform:EtOH 90:10 for 2 min (Caution! Chloroform is a carcinogen!). Centrifuge at 1800 g for 20 min, add the organic phase to 50 μL 100 mM HCl, evaporate to dryness under a stream of nitrogen, dissolve the residue in 75 μL mobile phase, wash with 25 μL n-hexane, inject a 20 μL aliquot of the lower phase. Urine. Mix 500 μL urine with 25 μL 500 μg/mL IS in MeOH, 500 μL 750 mM pH 5.0 sodium acetate buffer containing 40 mg/mL sodium metabisulfite, and 10 mg of a mixture of β-glucuronidase and arylsulfatase

(Limpet acetone powder Type I: Platela vulgata, Sigma). Heat at 37° for 2 h, add 200 mg sodium chloride, extract with 2 mL dichloromethane:isopropanol 90:10 for 90 s, centrifuge at 1800 g for 30 min, evaporate the organic phase under a stream of nitrogen. Dissolve the residue in 200 μL mobile phase, vortex for 15 s, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeOH:250 mM pH 5.0 sodium acetate buffer 30:70
Flow rate: 1.0
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 4.5
Internal standard: phenacetin (10)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Lanchote,V.L.; Ping,W.C.; Santos,S.R.C.J. Determination of antipyrine and metabolites in plasma of a patient with mild renal failure, *Ther.Drug Monit.*, **1997**, *19*, 705–710.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + 500 μL 100 mM NaOH + 10 mL benzene (CAUTION! Benzene is a carcinogen!), shake vigorously for 20 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 250 μL MeOH, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water:acetic acid 20:75:5
Flow rate: 1
Injection volume: 25
Detector: UV 254 for 7 min then UV 280

CHROMATOGRAM
Retention time: 8.0
Internal standard: antipyrine

OTHER SUBSTANCES
Extracted: 4-monomethylaminoantipyrine

KEY WORDS
plasma; antipyrine is IS

REFERENCE
Asmardi,G.; Jamali,F. High-performance liquid chromatography of dipyrone and its active metabolite in biological fluids, *J.Chromatogr.*, **1983**, *277*, 183–189.

SAMPLE
Matrix: saliva
Sample preparation: Add 100 μL phenacetin to 1 mL saliva, inject an aliquot.

HPLC VARIABLES
Column: 10 μm Spheri-10 RP-18 (Brownlee)
Mobile phase: MeCN:buffer 7.5:92.5 (The buffer was 100 mM sodium acetate containing 0.5% triethylamine, pH 6.6.)
Column temperature: 40
Flow rate: 3.5
Detector: UV 254

CHROMATOGRAM
Internal standard: phenacetin

KEY WORDS
pharmacokinetics; saliva

REFERENCE
Jorquera,F.; Almar,M.M.; Gonzalez-Sastre,M.; Suarez,I.; Gonzalez-Gallego,J. Accuracy of the one-sample method for determination of antipyrine clearance in elderly subjects, *J.Pharm.Biomed.Anal.*, **1996**, *15*, 7–11.

SAMPLE
Matrix: saliva
Sample preparation: 500 μL Saliva + 500 μL MeCN + 50 μL 400 μg/mL phenacetin, vortex, centrifuge at 5500 g for 1 min. Filter (4.5 μm) the supernatant and inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 7 μm Eicopack MA-ODS (Eicom, Kyoto)
Mobile phase: MeCN:20 mM pH 6.0 potassium phosphate buffer 30:70 containing 0.1% triethylamine
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 4.5
Internal standard: phenacetin (8.2)
Limit of detection: 100 ng/mL

REFERENCE
Echizen,H.; Nakura,M.; Ishizaki,I. Rapid and simple high-performance liquid chromatographic determination of saliva antipyrine for routine antipyrine test, *J.Chromatogr.*, **1990**, *526*, 296–299.

SAMPLE
Matrix: saliva
Sample preparation: 1 mL Saliva + 100 μL 2 M NaOH + 100 μL 400 μg/mL phenacetin in EtOH + 5 mL n-pentane:dichloromethane 50:50, extract on a whirlmixer for 15 s. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 10 μm Spheri-10 RP-18
Mobile phase: MeCN:100 mM sodium acetate:triethylamine 7.5:92:0.5, pH 6.6
Column temperature: 40
Flow rate: 3.5
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Internal standard: phenacetin

KEY WORDS
pharmacokinetics

REFERENCE
Jorquera,F.; Almar,M.M.; Pozuelo,M.; Sansegundo,D.; González-Sastre,M.; González-Gallego,J. Effects of aging on antipyrine clearance: Predictive factors of metabolizing capacity, *J.Clin.Pharmacol.*, **1995**, *35*, 895–901.

SAMPLE
Matrix: saliva
Sample preparation: Saliva + phenacetin + MeCN, vortex, centrifuge, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 4.6 5 μm ODS-Technosphere (HPLC Technology)
Mobile phase: MeCN:50 mM pH 6.0 sodium phosphate buffer 1:2
Flow rate: 1.5
Injection volume: 20
Detector: UV 260

CHROMATOGRAM
Internal standard: phenacetin
Limit of detection: 100 nM

REFERENCE
Perrett,D.; Ross,G.A. Rapid determination of drugs in biofluids by capillary electrophoresis. Measurement of antipyrine in saliva for pharmacokinetic studies, *J.Chromatogr.A*, **1995**, *700*, 179–186.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 40:60 containing 400 mM KCl
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 230

OTHER SUBSTANCES
Also analyzed: chlorpheniramine

REFERENCE
Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeOH:acetic acid:triethylamine:water 30:1.5:0.5:68
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM

Retention time: k' 2.60

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazo-

cine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 5.39 (A), 4.54 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone,

phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
also details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of an aqueous solution.

HPLC VARIABLES
Column: 150 × 3 4 μm Supersphere 100 RP18 endcapped (Bischoff)
Mobile phase: MeCN:20 mM pH 7.4 KH_2PO_4 10:90
Flow rate: 0.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 25

OTHER SUBSTANCES
Simultaneous: degradation products, radiation degradation products, o-hydroxyantipyrine, m-hydroxyantipyrine, p-hydroxyantipyrine

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Coolen,S.A.J.; Everaerts,F.M.; Huf,F.A. Characterization of ^{60}Co γ-radiation induced radical products of antipyrineby means of high-performance liquid chromatography, mass spectrometry, capillary zone electrophoresis, micellar electrokinetic capillary chromatography and nuclear magnetic resonance spectrometry, *J.Chromatogr.A*, **1997**, *788*, 95–103.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 1.70

OTHER SUBSTANCES
Also analyzed: amoxicillin, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

SAMPLE
Matrix: urine
Sample preparation: Condition a C18 AASP SPE cartridge (Varian) with 1 mL MeOH and 1 mL water. 100 μL Urine + 100 μL 50 mM pH 4.5 KH_2PO_4 + 20 mg limpet acetone powder (type I, contains β-glucuronidase and sulfatase, from Sigma), + 16 mg sodium metabisulfite, vortex for 15 s, heat at 40° with mild agitation for 3 h. Cool to room temperature, add 10 μL 1 mg/mL 4-dimethylaminoantipyrine in water, vortex, centrifuge at 500 g for 20 min. Remove 100 μL supernatant and add it to 900 μL pH 4.5 100 mM TRIS. Remove 500 μL and add it to 500 μL 50 mM pH 4.5 KH_2PO_4, add to SPE cartridge, wash with four 1 mL portions of 50 mM pH 4.5 phosphate buffer, allow to dry completely, elute with three 100 μL volumes of MeCN:dichloromethane 20:80. Evaporate eluate at 40° under vacuum while vortexing, reconstitute the residue in 100 μL 50 mM pH 4.5 phosphate buffer, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2 40 μm Corasil phenyl
Column: 100 × 4.6 5 μm Spherisorb phenyl
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeOH. A:B from 90:10 to 60:40 over 14 min, to 40:60 over 7 min, to initial conditions over 0.1 min, re-equilibrate for 7.9 min.
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 17.8
Internal standard: 4-dimethylaminoantipyrine (19.9)
Limit of quantitation: 2000 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
SPE

REFERENCE
Sarkar,M.A.; March,C.; Karnes,H.T. Solid phase extraction and simultaneous high performance liquid chromatographic determination of antipyrine and its major metabolites in urine, *Biomed.Chromatogr.*, **1992**, *6*, 300–304.

SAMPLE
Matrix: urine
Sample preparation: Inject a 100 μL aliquot directly.

HPLC VARIABLES
Guard column: 25 × 4 5 μm LiChrospher 100 RP 18
Column: 125 × 4 5 μm LiChrospher 60 RP Select B
Mobile phase: Gradient. A was 5 mM tetrabutylammonium phosphate in water. B was 5 mM tetrabutylammonium phosphate in MeOH. A:B from 90:10 to 87:13 over 10 min, to 67:33 over 16 min, to 60:40 over 9 min, to 0:100 over 10 min, maintain at 0:100 for 5 min, return to initial conditions over 5 min.
Flow rate: 0.7
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 23

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; dog

REFERENCE
Velic,I.; Metzler,M.; Hege,H.G.; Weymann,J. Separation and identification of phase I and phase II [^{14}C]antipyrine metabolites in rat and dog urine, *J.Chromatogr.B*, **1995**, *666*, 139–147.

Antitrypsin

Molecular formula: indeterminate
CAS Registry No.: 9041-92-3
Merck Index: 760

SAMPLE

Matrix: blood
Sample preparation: Dialyze 2 mL plasma against 30 mM pH 7.0 sodium phosphate buffer overnight, chromatograph on 20 mL Blue-Sepharose (equilibrated with 30 mM pH 7.0 sodium phosphate buffer) at 40 mL/h. Dialyze non-retained fraction (20-30 mL) against 30 mM pH 5.8 sodium acetate buffer overnight, chromatograph on 10 mL Red-Sepharose (equilibrated with 30 mM pH 5.8 sodium acetate buffer). Concentrate non-retained fraction (30-50 mL) to 2-3 mL with Amicon PM-10 and freeze dry, dissolve freeze-dried sample in 300 μL 10 mM pH 7.0 sodium phosphate buffer containing 100 mM NaCl, inject into HPLC.

HPLC VARIABLES

Column: 100 × 6 KB-column hydroxy-apatite (Koken)
Mobile phase: Gradient. A was 10 mM pH 7.0 sodium phosphate buffer containing 100 mM NaCl. B was 200 mM pH 7.0 sodium phosphate buffer containing 100 mM NaCl. A:B from 100:0 to 50:50 over 1 h.
Flow rate: 1
Detector: UV 280

CHROMATOGRAM

Retention time: 22.3

OTHER SUBSTANCES

Extracted: α1-acid glycoprotein

KEY WORDS

plasma

REFERENCE

Funae,Y.; Wada,S.; Imaoka,S.; Hirotsune,S.; Tominaga,M.; Tanaka,S.; Kishimoto,T.; Maekawa,M. Chromatographic separation of *α1*-acid glycoprotein from *α1*-antitrypsin by high-performance liquid chromatography using a hydroxyapatite column, *J.Chromatogr.*, **1986**, *381*, 149–152.

SAMPLE

Matrix: blood
Sample preparation: Prepare concentrate from plasma (procedure given).

HPLC VARIABLES

Column: superose-12 (Pharmacia)
Mobile phase: 500 mM NaCl
Flow rate: 0.5
Injection volume: 50
Detector: UV 280

KEY WORDS

plasma

REFERENCE

Burnouf,T.; Constans,J.; Clerc,A.; Descamps,J.; Martinache,L.; Goudemand,M. Biochemical and biological properties of an α_1-antitrypsin concentrate, *Vox Sang.*, **1987**, *52*, 291–297.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in PBS.

HPLC VARIABLES

Column: 600 × 7.5 Spheragel-TSK 3000 SW
Mobile phase: PBS
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM

Retention time: 16.4

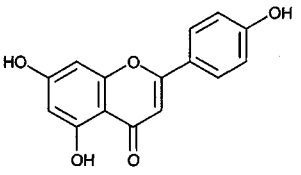
OTHER SUBSTANCES

Simultaneous: aggregates

REFERENCE

Glaser,C.B.; Busby,T.F.; Ingham,K.C.; Childs,A. Thermal denaturation of α 1-protease inhibitor. Stabilization by neutral salts and sugars, *Am.Rev.Respir.Dis.*, **1983**, *128*, 77–81.

Apigenin



Molecular formula: $C_{15}H_{10}O_5$
Molecular weight: 270.24
CAS Registry No.: 520-36-5
Merck Index: 773

SAMPLE

Matrix: tissue
Sample preparation: Homogenize epidermal cells in absolute EtOH. Evaporate the solvent, dissolve the residue in 100 μL absolute EtOH containing 2.4 μg/mL IS. Filter (0.20 μm nylon membrane), inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 2.1 Alltima C18 (Alltech)
Mobile phase: MeCN:water 48:52 containing 0.1% trifluoroacetic acid
Flow rate: 0.3
Injection volume: 5
Detector: UV 337

CHROMATOGRAM

Retention time: 3.76
Internal standard: quercetin (3.06)
Limit of detection: 1.15 ng

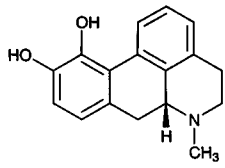
KEY WORDS

epidermal cells; mouse

REFERENCE

Li,B.; Robinson,D.H.; Birt,D.F. Evaluation of properties of apigenin and [G-3H]apigenin and analytic method development, *J.Pharm.Sci.*, **1997**, *86*, 721–725.

Apomorphine



Molecular formula: $C_{17}H_{17}NO_2$
Molecular weight: 267.33
CAS Registry No.: 58-00-4, 41372-20-7 (HCl)
Merck Index: 787

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 206.4

CHROMATOGRAM

Retention time: 8.962

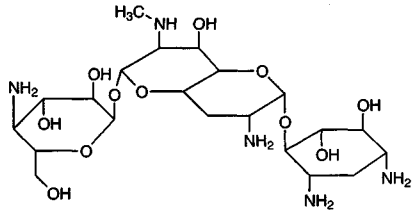
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Apramycin



Molecular formula: $C_{21}H_{41}N_5O_{11}$
Molecular weight: 539.58
CAS Registry No.: 37321-09-8
Merck Index: 792

SAMPLE

Matrix: fermentation solutions
Sample preparation: 5 mL Fermentation broth + 5 mL saturated aqueous solution of Tris + 20 mL MeCN, centrifuge at 3000 rpm for 10 min. Remove a 1 mL aliquot of the supernatant and add it to 3 mL 150 mM 2,4-dinitrofluorobenzene in MeOH, heat at 100° under a reflux condenser for 45 min, make up to 4 mL with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 200 × 4.6 10 μm LiChrosorb RP-8
Mobile phase: MeCN:water:acetic acid 55:45:0.15
Flow rate: 1.2
Injection volume: 20
Detector: UV 350

CHROMATOGRAM

Retention time: 9.56

OTHER SUBSTANCES

Extracted: kanamycin B, tobramycin

KEY WORDS

derivatization

REFERENCE

Harangi,J.; Deák,M.; Nánási,P.; Bacsa,G. Determination of the major factors of fermentation of the nebramycin complex by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1984**, *7*, 83–93.

SAMPLE

Matrix: reaction mixtures
Sample preparation: 50 μL Buffered reaction mixture + 50 μL isopropanol + 50 μL reagent, heat at 60° for 10 min, centrifuge at 1000 g for 2 min, immediately inject a 50 μL aliquot of the supernatant. (Reagent was 80 mM o-phthalaldehyde and 250 mM thioglycolic acid in 1 M boric acid, pH adjusted to 10.4 with 40% KOH.)

HPLC VARIABLES

Column: 100 × 5 Hypersil ODS
Mobile phase: A was MeOH:water:acetic acid 50:45:5 containing 5 g/L heptanesulfonic acid. B was MeOH:water:acetic acid 75:20:5 containing 5 g/L heptanesulfonic acid. A:B 70:30.
Flow rate: 2
Injection volume: 50
Detector: UV 330

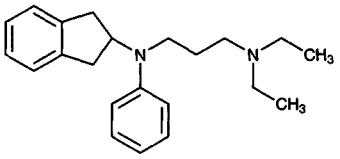
CHROMATOGRAM

Retention time: 20.5

REFERENCE

Lovering,A.M.; White,L.O.; Reeves,D.S. Identification of aminoglycoside-acetylating enzymes by high-pressure liquid chromatographic determination of their reaction products, *Antimicrob.Agents Chemother.*, **1984**, *26*, 10–12.

Aprindine



Molecular formula: $C_{22}H_{30}N_2$
Molecular weight: 322.49
CAS Registry No.: 37640-71-4, 33237-74-0 (HCl)
Merck Index: 793

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 16.967

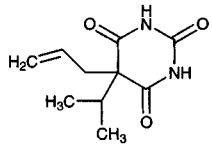
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Aprobarbital



Molecular formula: $C_{10}H_{14}N_2O_3$
Molecular weight: 210.23
CAS Registry No.: 77-02-1
Merck Index: 794
Lednicer No.: 1 268

SAMPLE

Matrix: blood
Sample preparation: Extract 20 μL with three 20 μL portions of acetone:diethyl ether 50:50. Combine the organic layer s and add 5 μL ethyl acetate, dry over 4 Å molecular sieve, evaporate to about 5 μL (mostly ethyl acetate), add 15 μg dansyl chloride, add 2 mg potassium carbonate, reflux for 2 h, dilute to 100 μL, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 500 × 2.5 30 μm pellicular C18
Mobile phase: Gradient. MeOH:water 0:100 for 8 min, to 65:35 over 20 min.
Flow rate: 1
Injection volume: 5
Detector: F ex 360 em 520

CHROMATOGRAM

Retention time: 16

OTHER SUBSTANCES

Extracted: barbital, heptabarbital

KEY WORDS

derivatization; whole blood

REFERENCE

Dünges,W.; Naundorf,G.; Seiler,N. High pressure liquid chromatographic analysis of barbiturates in the picomole range by fluorometry of their DANS-derivatives, *J.Chromatogr.Sci.*, **1974**, *12*, 655–657.

SAMPLE

Matrix: blood
Sample preparation: Mix plasma with an equal volume of MeCN, centrifuge at 10000 g, dilute supernatant with an equal volume of water, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 110 × 4.7 5 μm PartiSphere C18 (Whatman)
Mobile phase: MeCN:15 mM pH 7.0 phosphate buffer 30:70
Flow rate: 0.8
Injection volume: 50
Detector: UV 270 following post-column reaction. The column effluent flowed through a 6 m × 0.25 mm ID crocheted coil of PTFE tubing irradiated by an 8 W low-pressure mercury lamp to the detector.

CHROMATOGRAM

Retention time: 4.4

OTHER SUBSTANCES

Extracted: butethal, pentobarbital, secobarbital

KEY WORDS
plasma; post-column reaction; post-column photochemical derivatization

REFERENCE
Wolf,C.; Schmid,R.W. Enhanced UV-detection of barbiturates in HPLC analysis by on-line photochemical reaction, *J.Liq.Chromatogr.*, **1990**, *13*, 2207–2216.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 0.5 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN:water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 14.3

OTHER SUBSTANCES
Simultaneous: acetaminophen, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, diflunisal, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, testosterone, testosterone propionate, tranylcypromine, tripelennamine
Interfering: doxapram

KEY WORDS
details for purification of triethylamine in paper

REFERENCE
Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mm KH_2PO_4 + 5 mM 1-decanesulfonic acid 30:70, adjusted to pH 3.2 with 85% phosphoric acid
Flow rate: 1
Injection volume: 10
Detector: UV 214

CHROMATOGRAM
Retention time: 5.8
Internal standard: methyl paraben (7.0)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: allobarbital, barbital, butalbital, mephobarbital, pentobarbital, phenobarbital, secobarbital, talbutal, vinbarbital

KEY WORDS
stability-indicating

REFERENCE
Ibrahim,F.B. Simultaneous determination and separation of several barbiturates and analgesic products by ion-pair high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 2835–2851.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in mobile phase to a concentration of 50 μg/mL.

HPLC VARIABLES
Column: 250 × 4 β-cyclodextrin polymer-coated silica (Chromatographia 1993, 36, 373)
Mobile phase: MeOH:water 50:50
Flow rate: 0.6
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: k' 0.398

OTHER SUBSTANCES
Simultaneous: pentobarbital, amobarbital, butabarbital, butalbital, secobarbital, thiopental, phenobarbital

REFERENCE
Forgács,E.; Cserháti,T. Retention behaviour of barbituric acid derivatives on a β-cyclodextrin polymer-coated silicon column, *J.Chromatogr.A*, **1994**, *668*, 395–402.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine,

eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Aprotinin

Molecular formula: $C_{284}H_{432}N_{84}O_{79}S_7$
Molecular weight: 6511.53
CAS Registry No.: 9087-70-1
Merck Index: 796

SAMPLE

Matrix: blood, urine
Sample preparation: Prepare an affinity column with porcine pancreatic kallikrein bound to cyanogen bromide-activated Sepharose 4B according to the method of the manufacturer (Pharmacia). Use prolonged washing cycles of high and low pH. Immediately acidify urine to pH 2.0. Adjust pH of plasma to 8.3 with 100 mM pH 8.3 Tris-HCl buffer containing 500 mM NaCl. Adjust pH of urine to 8.3 with 2 M NaOH. Centrifuge sample at 4390 g for 10 min, pump onto affinity column, pump eluate twice onto column. Wash column with ten bed volumes 100 mM pH 8.3 Tris-HCl buffer containing 500 mM NaCl. Elute with 800 μL 1 M phosphoric acid and 800 μL 10 mM pH 2.2 phosphoric acid in 200 mM sodium perchlorate, mix, inject a 1000 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 7 μm LiChrosorb RP-18
Mobile phase: MeCN:200 mM pH 2.2 sodium perchlorate containing 10 mM phosphoric acid 30:70
Column temperature: 25
Flow rate: 1
Injection volume: 1000
Detector: UV 200

CHROMATOGRAM

Retention time: 9.3
Limit of detection: 50 ng/mL

KEY WORDS

plasma; affinity chromatography

REFERENCE

Raspi,G.; Lo Moro,A.; Spinetti,M. High-performance liquid chromatographic method for the determination of aprotinin in body fluids, *J.Chromatogr.*, **1990**, *525*, 426–432.

SAMPLE

Matrix: formulations
Sample preparation: Dilute (if necessary) to a concentration of 500 μg/mL, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelco LC-304 (pore size 30 nm)
Mobile phase: MeCN:31 mM sodium perchlorate 22:78
Flow rate: 0.5
Injection volume: 20
Detector: UV 214

CHROMATOGRAM

Retention time: 35
Limit of detection: 5000 ng/mL

REFERENCE

Dimov,N.; Simeonov,S. Purity evaluation of aprotinin by high performance liquid chromatography, *Biomed.Chromatogr.*, **1993**, *7*, 146–148.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 100-1000 μL aliquot of buffer solution containing aprotinin.

HPLC VARIABLES

Column: 250 × 4.6 7 μm LiChrosorb RP-18
Mobile phase: MeCN:10 mM phosphoric acid in 200 mM sodium perchlorate 30:70
Column temperature: 25
Flow rate: 1
Injection volume: 100-1000
Detector: UV 200

CHROMATOGRAM

Retention time: 9.3
Limit of detection: 600 ng/mL

REFERENCE

Raspi,G.; Lo Moro,A.; Spinetti,M.; Molinari,M. Determination of aprotinin by titration with bovine trypsin with end-point detection by high-performance liquid chromatography, *Analyst*, **1989**, *114*, 1017–1019.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: Vydac C4 (code 214TP54)
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid and 2% ammonium sulfate. B was 0.07% trifluoroacetic acid in MeCN. A:B from 95:5 to 75:25 over 20 min.
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM

Retention time: 15.8

OTHER SUBSTANCES

Simultaneous: degradation products

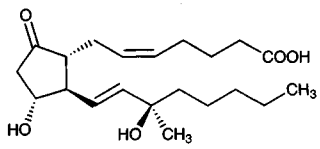
KEY WORDS

cow

REFERENCE

Vinther,A.; Bjorn,S.E.; Sorensen,H.H.; Soeberg,H. Identification of aprotinin degradation products by the use of high-performance capillary electrophoresis, high-pressure liquid chromatography and mass spectrometry, *J.Chromatogr.*, **1990**, *516*, 175–184.

Arbaprostil



Molecular formula: $C_{21}H_{34}O_5$
Molecular weight: 366.50
CAS Registry No.: 55028-70-1
Merck Index: 808
Lednicer No.: 3 8

SAMPLE

Matrix: blood

Sample preparation: Condition a 3 mL 200 mg and a 1 mL 100 mg Bond-Elut C18 SPE cartridge with 4 mL MeOH and 4 mL water. 3 mL Plasma + 12 μL 32.5 ng/mL IS in MeCN + 300 μL 5% formic acid, vortex, centrifuge at 4° at 1500 g for 15 min, add the supernatant to the 3 mL SPE cartridge, wash with two 2 mL portions of water, wash with two 2 mL portions of MeOH:water 10:90, wash with 2 mL toluene, elute with 1 mL ethyl acetate. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute with 610 μL MeOH, vortex for 30 s, add 1.42 mL 0.01% formic acid, inject a 1.8 mL aliquot onto a 30 × 4.6 5 μm Brownlee RP-8 column and elute to waste with MeCN:water:formic acid 40:60:0.01 at 2 mL/min, after 2.5 min backflush the contents of this column onto a 250 × 4.6 5 μm Supelcosil LC-18 column eluted with MeCN:water:formic acid 40:60:0.01 at 2 mL/min, after about 6.5-7 min collect a fraction containing the prostaglandins. Dilute this fraction with an equal volume of water, add to the 1 mL SPE cartridge, wash with 1 mL hexane, elute with two 500 μL portions of ethyl acetate. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 250 μL 100 μg/mL panacyl bromide in THF:MeCN 20:80, vortex for 30 s, add 3 μL N,N-diisopropylethylamine, heat at 40° for 1 h (Anal. Chem. 1984, 56, 1866). Evaporate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 230 μL isooctane:ethylene dichloride:isopropanol 70:30:1, sonicate for 10 min, inject a 200 μL aliquot onto column A and elute to waste with mobile phase A, after 3 min divert the effluent from column A onto column B and elute both to waste, after 1.5 min remove column A from the circuit, continue to elute column B to waste with mobile phase A, after 7.5 min collect the effluent from column B in a 2.2 mL sample loop, after 2 min inject the contents of this sample loop onto column C with mobile phase B, elute column C with mobile phase B, monitor the effluent from column C. (Synthesize panacyl bromide (p-(9-anthroyloxy)phenacyl bromide) as follows. Add 3.04 g benzyltrimethylammonium dichloroiodate to a solution of 500 mg 4'-hydroxyacetophenone in 50 mL dichloroethane and 20 mL MeOH, reflux for 10 h, remove the solvent by distillation, add 20 mL 5% sodium bisulfite to the residue, extract four times with 40 mL portions of ether, dry over anhydrous magnesium sulfate, evaporate to dryness under reduced pressure to give p-hydroxyphenacyl chloride (mp 151-152°) (Synthesis 1988, 545). Purify p-hydroxyphenacyl chloride by suspending 100 g in 1 L boiling toluene, filter, cool to obtain white crystals of p-hydroxyphenacyl chloride. Repeat this process a number of times to obtain more pure product. Reflux 10 g 9-anthracenecarboxylic acid in 150 mL redistilled thionyl chloride for 2 h, evaporate to dryness under reduced pressure at 30°, dissolve the residue in 150 mL dry toluene containing 11.5 g p-hydroxyphenacyl chloride, reflux for 2 h, evaporate to dryness under reduced pressure, recrystallize from 200 mL hot MeCN to give p-(9-anthroyloxy)phenacyl chloride as deep yellow crystals (mp 159.8-161.6°). Dissolve 2.5 g p-(9-anthroyloxy)phenacyl chloride in 25 mL THF:MeCN 20:80, add 8 g anhydrous LiBr, reflux briefly, cool to room temperature, filter, wash the solid with water to obtain p-(9-anthroyloxy)phenacyl bromide as deep yellow crystals (mp 173.3-173.6°) (Anal. Biochem. 1987, 165, 220).)

HPLC VARIABLES

Column: A 10 × 4.6 Co:Pell PAC; B 150 × 4.6 6 μm Zorbax CN; C 240 × 4.6 6 μm Zorbax Sil

Mobile phase: A Hexane:dichloromethane:isopropanol 70:30:1; B Hexane:dichloromethane:THF:isopropanol 60:20:20:1
Flow rate: 1
Injection volume: 200
Detector: F ex 375 em 470

CHROMATOGRAM
Retention time: 36 (arbaprostil), 39.5 (15S epimer)
Internal standard: 5,6-trans-(15R)-15-methylprostaglandin E_2 (U-67205) (37.5)
Limit of quantitation: 10 pg/mL

KEY WORDS
derivatization; SPE; plasma; column-switching; normal phase

REFERENCE
Pullen,R.H.; Cox,J.W. Determination of (15*R*)- and (15*S*)-15-methylprostaglandin E2 in human plasma with picogram per milliliter sensitivity by column-switching high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *343*, 271–283.

SAMPLE
Matrix: formulations
Sample preparation: 1 mL Formulation + IS + 500 μL 2% phosphoric acid, mix, add 10 mL ethyl ether:chloroform 80:20, extract, centrifuge. Remove 8 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 1 mL 2.5 mg/mL p-nitrophenacyl bromide in MeCN, add 500 μL 12.5 μL/mL N,N-diisopropylethylamine in MeCN, vortex briefly, heat at 40° for 30 min, evaporate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 2 mL mobile phase, vortex, inject a 5-25 μL aliquot.

HPLC VARIABLES
Column: μPorasil silica gel
Mobile phase: MeCN:dichloromethane:water 30:70:0.5
Flow rate: 1.5
Injection volume: 5-25
Detector: UV 254

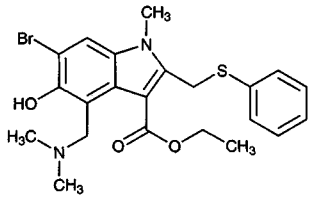
CHROMATOGRAM
Retention time: 6.8
Internal standard: 17β-hydroxy-17-methyl-4-androstene-3,11-dione (5.8)

OTHER SUBSTANCES
Simultaneous: s-epimer

KEY WORDS
derivatization; injections; normal phase; siliconise glassware with Surfasil (Pierce)

REFERENCE
Peng,G.W.; Sood,V.K. Liquid chromatographic assay of arbaprostil, *J.Liq.Chromatogr.*, **1983**, *6*, 1499–1511.

Arbidol



Molecular formula: $C_{22}H_{25}BrN_2O_3S$
Molecular weight: 477.42
CAS Registry No.: 131707-25-0, 131707-23-8 (HCl)

SAMPLE

Matrix: blood

Sample preparation: Vortex thawed plasma for 15 s, centrifuge at14 000 rpm for 1 min. Add 250 μL saturated sodium hydrogen carbonate to 1 mL plasma. Add 100 μL 5 μg/mL IS in MeCN and 5 mL MTBE. Shake at 50 rpm for 10 min and centrifuge at 4000 rpm for 10 min. Evaporate organic phase at 35° under a gentle flow of nitrogen. Reconstitute the residue in 200 μL mobile phase, centrifuge at 14000 rpm for 5 min. Add 200 μL hexane, mix for 15 sec, centrifuge at 14000 rpm for 3 min. Inject a 100 μL aliquot of the aqueous phase. (Protect sample from light!)

HPLC VARIABLES

Guard column: 4 × 4 5 μm LiChrospher RP 8
Column: 250 × 4.6 5 μm LiChrosorb RP 8
Mobile phase: MeOH:buffer 70:30 adjusted to pH 3.0 with orthophosphoric acid (Buffer was 5 mM heptanesulfonic acid containing 50 mM ammonium perchlorate and 1.32% (v/v) triethylamine.)
Column temperature: 35
Flow rate: 1.2
Injection volume: 100
Detector: UV 315

CHROMATOGRAM

Retention time: 5.3-5.4
Internal standard: SI 5 (G.D.Searle) (8.5-8.8)
Limit of quantitation: 5 ng/mL

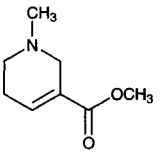
KEY WORDS

plasma

REFERENCE

Metz,R.; Muth,P.; Ferger,M.; Kukes,V.G.; Vergin,H. Sensitive high-performance liquid chromatographic determination of arbidol, a new antiviral compound in human plasma, *J.Chromatogr.A*, **1998**, *810*, 63–69.

Arecoline



Molecular formula: $C_8H_{13}NO_2$
Molecular weight: 155.20
CAS Registry No.: 63-75-2, 300-08-3 (HBr)
Merck Index: 815

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 207.5

CHROMATOGRAM

Retention time: 3.1

KEY WORDS

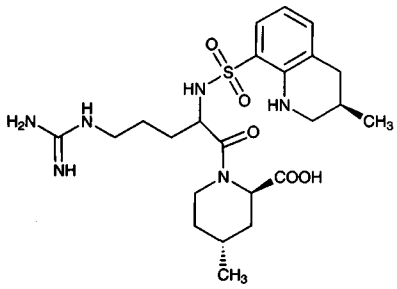
whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Argatroban

Molecular formula: $C_{23}H_{36}N_6O_5S$
Molecular weight: 508.64
CAS Registry No.: 74863-84-6
Merck Index: 816



SAMPLE
Matrix: solutions

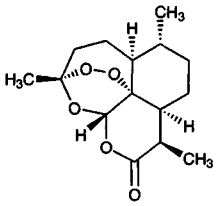
HPLC VARIABLES
Column: 250 × 4.6 Rainin 300Å C-18
Mobile phase: MeOH:water 33:67 containing 10 mM ammonium acetate
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 153 (R), 163 (S)

KEY WORDS
chiral

REFERENCE
Rawson,T.E.; VanGorp,K.A.; Yang,J.; Kogan,T.P. Separation of 21-(R)- and 21-(S)-argatroban: solubility and activity of the individual diastereoisomers [letter], *J.Pharm.Sci.*, **1993**, *82*, 672–673.

Artemisinin



Molecular formula: $C_{15}H_{22}O_5$
Molecular weight: 282.34
CAS Registry No.: 63968-64-9
Merck Index: 856

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 4 mL n-chlorobutane, vortex for 30 s, centrifuge at 5000 rpm for 15 min, freeze in acetone/dry ice. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL MeOH, add 400 μL 0.2% NaOH, mix, heat at 45° for 30 min, cool, add 50 μL 100 mM acetic acid in MeOH, inject a 200 μL aliquot.

HPLC VARIABLES

Column: 100 mm long 5 μm LiChrosorb C18
Mobile phase: MeOH:10 mM pH 4.5 phosphate buffer 45:55
Flow rate: 0.8
Injection volume: 200
Detector: UV (wavelength not specified)

CHROMATOGRAM

Retention time: 10
Limit of detection: 2.5 ng/mL

KEY WORDS

serum; pharmacokinetics; derivatization

REFERENCE

Titulaer,H.A.C.; Zuidema,J.; Kager,P.A.; Wetsteyn,J.C.F.M.; Lugt,C.B.; Merkus,F.W.H.M. The pharmacokinetics of artemisinin after oral, intramuscular and rectal administration to volunteers, *J.Pharm.Pharmacol.*, **1990**, *42*, 810–813.

SAMPLE

Matrix: blood
Sample preparation: Condition a 4 mm diameter Empore C18 SPE membrane with 0.5 mL MeOH and 0.5 mL water, do not allow to dry. Centrifuge 1 mL serum, add to SPE membrane, wash with 300 μL water, elute with 100 μL MeCN:water 65:35, inject a 50 μL aliquot of the eluate.

HPLC VARIABLES

Column: 150 × 2 5 μm Ultrasphere ODS
Mobile phase: MeCN:water 50:50
Flow rate: 0.3
Injection volume: 50
Detector: Chemiluminescence in a fluorescence detector with no light source emission wavelength 425 nm. The effluent from the column mixed with reagent pumped at 0.5 mL/min and flowed through a convoluted mixing coil (1.1 mL dead volume) to the detector. (Reagent was 15 μg/mL luminol and 30 μg/mL hematin in 100 mM NaOH. Let stand for 30 min before use. Protect from light. Prepare daily.)

CHROMATOGRAM

Retention time: 7
Internal standard: dihydroartemisinin (5)

Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Noninterfering: arteether, artemether

KEY WORDS
serum; post-column reaction; SPE

REFERENCE
Green,M.D.; Mount,D.L.; Todd,G.D.; Capomacchia,A.C. Chemiluminescent detection of artemisinin. Novel endoperoxide analysis using luminol without hydrogen peroxide, *J.Chromatogr.A*, **1995**, *695*, 237–242.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 250 μL saturated NaCl solution, vortex for 5 s, add 5 mL isooctane:1-chlorobutane 45:55, vortex for 3 min, centrifuge at 1440 g for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 50 μL EtOH:water 50:50, let stand at 4° for 18 h, deoxygenate with a stream of nitrogen at 5 mL/min for 2 min (J. Chromatogr. 1983, 256, 323), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Lichrosphere 100 CN
Mobile phase: MeCN:50 mM acetic acid 15:85 adjusted to pH 5.0 with 1 M NaOH
Column temperature: 30
Flow rate: 1.5
Injection volume: 20
Detector: E, Bioanalytical Systems Model 200A, glassy carbon electrode, Ag/AgCl reference electrode, operated in reductive mode under a helium atmosphere

CHROMATOGRAM
Retention time: 16.1
Internal standard: artemisinin

OTHER SUBSTANCES
Extracted: artemether, dihydroartemisinin

KEY WORDS
plasma; treat glassware with 5% dichlorodimethylsilane; artemisinin is IS

REFERENCE
Navaratnam,V.; Mansor,S.M.; Chin,L.K.; Mordi,M.N.; Asokan,M.; Nair,N.K. Determination of artemether and dihydroartemisinin in blood plasma by high-performance liquid chromatography for application in clinical pharmacological studies, *J.Chromatogr.B*, **1995**, *669*, 289–294.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL 100 mg Bond-Elut phenyl SPE cartridge with 1 mL MeOH and 1 mL 1 M acetic acid. Add 1 mL plasma to the SPE cartridge, wash with two 1 mL portions of 1 M acetic acid, wash with 1 mL MeOH:1 M acetic acid 20:80, elute with two 1 mL portions of ethyl acetate:butyl chloride 20:80. Remove any portion of aqueous phase from the eluate and evaporate the organic portion to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, inject a 50-75 μL aliquot.

HPLC VARIABLES
Guard column: Symmetry C8 (Waters)

Column: 150 × 3.9 5 μm Symmetry C8 (Waters)
Mobile phase: MeCN:buffer 50:50 (Buffer was 40 mL 1 M acetic acid and 60 mL 1 M sodium acetate per liter, pH 4.8.)
Flow rate: 0.7
Injection volume: 50-75
Detector: UV 290 following post-column reaction. The column effluent mixed with 1.2 M KOH MeOH:water 90:10 pumped at 0.3 mL/min and the mixture flowed through a 1 mL reaction coil (Waters) at 69° to the detector.

CHROMATOGRAM
Retention time: 13

OTHER SUBSTANCES
Extracted: artesunic acid, α-dihydroartemisinin, β-dihydroartemisinin

KEY WORDS
artemisinin is IS; post-column reaction; plasma; SPE

REFERENCE
Batty,K.T.; Davis,T.M.; Thu,L.T.; Binh,T.Q.; Anh,T.K.; Ilett,K.F. Selective high-performance liquid chromatographic determination of artesunate and α- and β-dihydroartemisinin in patients with falciparum malaria, *J.Chromatogr.B*, **1996**, *677*, 345–350.

SAMPLE
Matrix: blood, saliva
Sample preparation: 1.5 mL Plasma or saliva + 500 μL 0.9% NaCl + 2.5 mL ethyl acetate, vortex, centrifuge at 2800 rpm for 3 min, repeat extraction twice more. Combine the organic layers and evaporate them to dryness under a stream of air at room temperature, reconstitute the residue in 100 μL EtOH, add 400 μL 0.2% NaOH, heat at 50° for 30 min, cool rapidly in water, wash twice with 500 μL aliquots of ethyl acetate, centrifuge, evaporate traces of ethyl acetate with a stream of air at room temperature, add 40 μL 2.5 M acetic acid in EtOH, make up to 500 μL with MeOH:water 20:80, inject a 200 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm LiChrosorb RP-18
Mobile phase: MeOH:water 40:60 containing 10 mM Na_2HPO_4-NaH_2PO_4
Column temperature: 35 ± 1
Flow rate: 1.3
Injection volume: 200
Detector: UV 260

CHROMATOGRAM
Retention time: 12
Limit of detection: 2.5 ng/mL

KEY WORDS
derivatization; plasma; pharmacokinetics

REFERENCE
Zhao,S. High-performance liquid chromatographic determination of artemisinine (Qinghaosu) in human plasma and saliva, *Analyst*, **1987**, *112*, 661–664.

SAMPLE
Matrix: leaves
Sample preparation: Dry Artemisia leaves at 40° for 24 h, crush, reflux with 100 mL hexane for 15 min, filter, evaporate hexane to dryness under vacuum at 40°. Add 25 mL

MeCN to the residue, sonicate, filter (0.45 μm). Add 100 μL filtrate to 100 μL 1 mg/mL acetophenone in MeCN, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 Brownlee C18
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:buffer 55:45 (Buffer was 8.3 g sodium acetate and 4 mL glacial acetic acid in 1 mL water, pH 5.1.)
Flow rate: 0.45
Injection volume: 50
Detector: UV 289 following post-column derivatization with 1 M KOH in MeOH:water 9:1 at 0.2 mL/min. The mixture flowed through a 4.4 × 0.5 mm (sic) knitted PTFE capillary held at 70°.

CHROMATOGRAM

Retention time: 16.5
Internal standard: acetophenone (11.5)
Limit of detection: 25 ng

KEY WORDS

post-column reaction

REFERENCE

ElSohly,H.N.; Croom,E.M.; ElSohly,M.A. Analysis of the antimalarial sesquiterpene artemisinin in *Artemisia annua* by high-performance liquid chromatography (HPLC) with postcolumn derivatization and ultraviolet detection, *Pharm.Res.*, **1987**, *4*, 258–260.